

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :)

Akira ASAKURA *et al.*)

Serial No.: 09/470,667)

Filed: December 22, 1999)

For: **NOVEL ALCOHOL/ALDEHYDE
DEHYDROGENASES**)

Examiner: M. Walicka

Art Unit: 1652

Commissioner for Patents
Washington, D.C. 20231

FIRST DECLARATION OF DR. MASAKO SHINJOH UNDER 37 C.F.R. § 1.132

Sir:

I, Masako Shinjoh, a citizen and resident of Japan, hereby declare as follows:

1. I am employed by Nippon Roche Research Center of Nippon Roche K.K., Kajiwara 200, Kamakura-shi, Kanagawa-ken 247-8530, Japan (hereafter "NRKK"). I currently hold the position of genetic engineer at NRKK. A copy of my *curriculum vitae* is attached as Exhibit 1.
2. I am a coinventor of U.S. patent application No. 09/470,667 (the '667 application). The present application discloses and claims, in part, recombinant polypeptides that have alcohol/aldehyde

dehydrogenase activity, recombinant expression vectors containing DNA sequences that encode such polypeptides, recombinant organisms containing such expression vectors, processes for producing recombinant enzymes having alcohol/aldehyde dehydrogenase activity, and processes for producing, *e.g.*, 2-keto-L-gulonic acid (2-KGA).

3. As set forth in more detail in the SECOND DECLARATION OF DR. MASAKO SHINJOH UNDER 37 CFR §1.132 filed concurrently herewith, after reviewing the Sequence Listing filed with the '667 application, how the nucleotide and amino acid sequences that make up the Sequence Listing were incorporated into the '667 application, and the original nucleotide printouts from the sequencing machine used to read the experimentally derived nucleotide sequences, I have come to the conclusion that SEQ ID NOs:1, 3, and 7 each contain a single base (SEQ ID NOs:1 and 3) or a single amino acid (SEQ ID NO:7) error that arose through typing errors.
4. By way of background, SEQ ID NOs: 1 and 3, as disclosed in the '667 application, were derived from chromosomal DNA obtained from a cell culture of *Gluconobacter oxydans* strain DSM No. 4025. SEQ ID NOs: 1 and 3 encode subunits (Enzyme A and Enzyme A",

respectively) of an alcohol/aldehyde dehydrogenase ("AADH"). AADH is used to produce, e.g., 2-keto-L-gluconic acid (2-KGA), an intermediate in the production of L-ascorbic acid (vitamin C). SEQ ID NO:7 is the amino acid sequence deduced from the nucleotide sequence of SEQ ID NO:3.

5. To confirm my belief that typographical errors were introduced into SEQ ID NOs:1, 3, and 7 of the '667 application, and that such errors would have been readily determined by one skilled in this art because of the public availability of the starting material (i.e., the same cell line used to generate the nucleotide sequence from which SEQ ID NOs:1 and 3 were determined in the '667 application), I have supervised and coordinated NRKK's attempt to confirm the errors in the nucleic acid sequences of SEQ ID NOs:1 and 3, and the amino acid sequence deduced from SEQ ID NO:3 and set forth as SEQ ID NO:7 in the '667 application.
6. On June 6, 2000, at my direction Ms. Ayano Makino-Matsubara, who works in the NRKK Export/Import & Purchasing department, sent an order letter, via facsimile, to the International Depository Authority, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH ("DSMZ"), a publicly available cell depository, requesting a sample of *Gluconobacter oxydans* strain DSM 4025,

the same strain used to clone and sequence the polynucleotide sequences identified in the '667 application as SEQ ID NOs:1 and 3. A copy of the order letter to the DSMZ is attached as Exhibit 2. Accompanying the order letter was a purchase request from NRKK (attached as Exhibit 3) and Form DSMZ-BP/13 (a REQUEST FOR FURNISHING SAMPLES OF DEPOSITED MICROORGANISM), which is attached as Exhibit 4. On the same day, Ms. Makino-Matsubara also sent to DSMZ, by common mail carrier, hard copies of the order letter, purchase order, and Form DSMZ-BP/13 transmitted via facsimile earlier in the day. (See Exhibit 5).

7. On June 19, 2000, I received a package from DSMZ postmarked June 14, 2000. The package contained an Invoice (No. 2002963 (Exhibit 6)), an ampoule labeled as containing lyophilized cells of DSM 4025, a delivery slip (no. 2002963 (Exhibit 7)), directions for cultivating the reconstituted DSM 4025 cells (Exhibit 8), and a copy of a RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT, Form DSM-BP/4 (Exhibit 9). I also took photographs of the contents of the package. Two of these photographs are enclosed as Exhibits 10 and 11.

8. On the same day I received the labeled ampoule containing the lyophilized DSM 4025 cells, I placed the ampoule in a refrigerator in

Building 27, room 524 of NRRC for storage until I could finalize arrangements for the cloning and sequencing of chromosomal DNA derived from these cells. This refrigerator is accessible only by authorized personnel of NRKK.

9. On August 11, 2000, I also took photographs of the refrigerator containing the labeled ampoule. Three representative photographs are attached as Exhibits 12, 13, and 14. I also sent an e-mail to Mr. Mashita on August 11, 2000 to confirm that the DSM 4025 cells would be forwarded to Sawady on August 16, 2000 (a copy of the original email in Japanese is enclosed as Exhibit 15 and its translation in English as Exhibit 16).
10. On August 16, 2000, I retrieved the labeled ampoule containing the lyophilized DSM 4025 cells from the refrigerator in Building 27. I then packaged the ampoule for transport, and deposited it with a local delivery service (Takkyubin) for overnight delivery to Mr. Masao Mashita, sales director of K. K. Sawady Technology, 1-29-10, Mamo-cho, Itabashi-ku, Tokyo, 174-0063, Japan ("Sawady"). A copy of the Takkyubin delivery slip and a certified English translation of the relevant part thereof are attached as Exhibits 17 and 18, respectively.

11. Along with the ampoule, the package I forwarded to Mr. Mashita contained a letter providing sequence information for generating forward and reverse primers to be used by Sawady in the cloning and sequencing of the relevant parts of SEQ ID NOs:1 and 3.
12. In sum, the ampoule containing the lyophilized DSM 4025 cells that I received from DSMZ on June 19, 2000 was the same ampoule that I forwarded to Mr. Mashita at Sawady on August 16, 2000.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: September 18, 2002

Masako Shinjoh
Masako Shinjoh